

# MULTIPLE HOMOGENIZER

FOR RAPID SAMPLE PREPARATION IN IMMUNOASSAYS AND ELECTROPHORESIS



## Rapid Preparation Of Tissue Samples For Immunoassays And Electrophoresis

Reference: R. H. ffrench-Constant/A. L. Devonshire *Biochemical Genetics Vol. 25 Nos. 7/8 1987.*

The Multiple Homogenizer (originally designed for insect samples) allows simultaneous preparation of 96 tissue samples, reducing homogenization time by a factor of 10-fold. Up to 3000 samples for immuno-assay analysis may be prepared by one person daily.

Earlier labour-intensive and time-consuming methods have employed the use of standard micro-centrifuge tubes with close fitting glass rods. These methods proved to be the limitation in sample preparation when quantifying the activity of the enzyme responsible for the resistance to insecticides in aphids.

The illustration shows the precision component parts and the method of assembly. When buffer and samples have been loaded into the wells of the microplate the multi-pestle is inserted and rotated manually in an orbital motion.

The volume lost after this process regardless of homogenization volume was only 5µl when the pestles were extracted carefully. Thus 50% and 90% of sample was recovered reliably when homogenized in 10µl and 50µl respectively.

Other items of equipment for the analytical process include a special electrophoresis gel comb (corresponding to the pitches of the microplate), an 8-position multi-pipette, electrophoresis cabinet and power supply.

## A Multiple Homogenizer for the rapid preparation of samples for immunoassays and electrophoresis

by Alan Devonshire.

Studies of the population genetics of small organisms, such as insects, which rely on electrophoretic or immunoassay techniques, require analysis of many individuals to be representative. Samples of insects for electrophoretic analysis have been homogenized by various homogenizers such as polypropylene microcentrifuge tubes with close-fitting glass rods. However, homogenization of single insects followed by loading of gels with microsyringes is the most labour-intensive and time-consuming part of the assay procedure. When an immunoassay was developed to quantify the activity of the enzyme responsible for resistance to insecticides in aphids the limitations of sample preparation became even more evident.

A multiple homogenizer was therefore designed to fit into a standard immunoplate, allowing 96 individual samples to be homogenized simultaneously for immunoassay or electrophoresis (ffrench-Constant & Devonshire, *Biochemical Genetics*, 25, 493-499 (1987)).

The homogenizer is made from a 10mm thick Perspex backing plate of similar dimensions to an immunoplate, into which 96 Perspex rods are inserted at 9mm spacing, to correspond precisely with the 96 wells of the immunoplate. The tips of the rods are ground flat, to rest uniformly on the bases of the flat-bottomed wells.

Samples are loaded into buffer in the wells of the immunoplate and homogenized by inserting and manually rotating the homogenizer. Rods of 4mm diameter are optimal, both for efficient homogenization and to leave a useful volume (200 µl) in the wells when assembled.

The volume "lost" during homogenization, quantified by placing a range of volumes (10-200µl of PBS/Tween plus sucrose and bromocresol purple) in separate eight-well columns of an immunoplate and determining the volume recoverable from all eight wells with the multipipette after insertion, rotation, and removal of the homogenizer rods, was only 5µl regardless homogenisation volume, provided that the homogenizer was extracted carefully. Thus 50% and 90% of the sample was recovered reliably when homogenized in 10 µl and 50 µl, respectively, covering the range of volumes typically used in electrophoresis of small organisms.

Combs with wells at 4.5 or 9mm spacing are required if samples are to be loaded directly from an immunoplate into the wells of an electrophoresis gel using an eight-channel multipipette. Purpose-made combs (now available from Burkard Scientific) with 4.5mm spacing enabled 14 samples to be loaded onto each gel of a Pharmacia GE-2/4 apparatus (3mm thick and 72mm wide) from two rows of an immunoplate using only seven tips of the eight-channel pipette twice to fill alternate wells, so that 56 samples could be analyzed simultaneously in a single "run" in one electrophoresis tank. Samples were also loaded successfully by multipipette into the wells of a gel cast in Hoefer equipment (the 15-well combs. No. SE 511-15, have 9.1mm spacing) even though the pipette tips were too large to reach into the wells of the 1.5mm tick gel.

Insects ranging from whitefly (50µg) to houseflies (20mg) were successfully homogenized, although quantitative assessments of efficiency were made only on aphids (ca. 400µg), using the immunoassay for the enzyme responsible for insecticide resistance. The mean enzyme activity extracted from individual aphids by the multiple homogenizer was significantly greater ( $P < 0.001$ ) and less variable ( $P < 0.001$ ) than that prepared in microcentrifuge tubes.

Besides homogenizing more effectively, sample preparation time is approximately one tenth of that needed for individual homogenization in microcentrifuge tubes. Other time-saving aspects of the system include ease of sample loading data recording for the shallow and numbered wells of the immunoplate, storage of samples in plates stacked in a freezer, and the use of multiple pipettes for all liquid handling. The availability of centrifuges for microplates provides the opportunity to remove tissue debris before analysis.

With the growth of immunological assays in surveying and diagnostic studies, this rapid sample preparation technique should have many biological and medical applications for tissues compatible with the usable volume (10-200µl) of the plate wells. The technique is well suited to electrophoretic studies provided that appropriate combs are available. Assuming no other constraints on comb well spacing, manufacturers should consider adopting a standard spacing of 4.5 or 9mm so that multiple pipettes can be used more extensively in sample preparation and loading.

## Ordering Information

Product Code	Description
BS00253	Multiple Homogenizer
BS01071	Gel Comb
BS01072	Multi-Pipette
BS01073	Electrophoresis Cabinet
BS01074	Power Supply Unit

The company reserves the right to make changes to the specification without notice.

